Grant# DK64540

Center Director: Scott J. Hultgren, Ph.D.

Center Overview

On a global basis, urinary tract infections (UTIs), which are among the most common infectious diseases in the United States, occur -150 million times annually and account for more than $6 billion in direct health care costs. UTIs primarily affect women because of the female- anatomy and the ascending nature of the disease. The Gram-negative bacterium Escherichia coli is the most common cause of these infections which account for significant morbidity. In addition, to the original infection one of the major clinical problems is recurrence. A woman treated for an uncomplicated UTI has a 25-50% chance of developing a recurrent infection. It has generally been assumed that UTIs are caused by non-invasive bacteria, that they are acute in nature, and are self-limited. It has also been assumed that recurrent infections are due to re-inoculation of the urinary tract with E.coli from an intestinal or vaginal reservoir. Recent evidence suggests that this dogma may in some cases be incorrect and misleading and could be interfering with the proper evaluation and treatment of these infections. This proposal seeks to elucidate the molecular and epidemiologic basis of acute and recurrent UTIs. Special emphasis will be placed on better defining the epidemiology of UTIs, determining the presence of persistent bacterial bladder and vaginal reservoirs following acute symptomatic UTI in women and elucidating the molecular factors involved in the host-pathogen interaction. The temporal, associations between asymptomatic and symptomatic bladder infection and vaginal colonization in the clinical setting will be assessed. Molecular characterization of the virulence determinants in well-characterized uropathogenic strains will be elucidated by blending a powerful genetic system with functional genomics, defined in vitro and murine models, biochemistry, cell biology, and high resolution electron microscopy. The mechanistic details of how the bladder responds to infection, and how specific virulence factors affect this host response will be determined using microarray, quantitative RT-PCR and laser capture microdissection. This proposal represents an intricate and integrated network between three projects and will lead to a new understanding of the host-pathogen interactions that occur throughout the infectious cycle including the host defense response in the bladder and the virulence mechanisms by which bacterial pathogens subvert these defenses. Results of this research could revolutionize the way UTIs are evaluated and lead to new and better ways to treat and prevent this infection that primarily affects women.

Principal Investigator: Scott J. Hultgren, Ph.D.

Project 1: Host-Pathogen Interactions in Acute and Chronic UTI

Millions of women suffer from recurrent urinary tract infections (UTIs) every year despite current antimicrobial treatments. UTIs are usually caused by the Gram-negative bacterium Escherichia coli. The Gram positive pathogen Enterococcus faecalis is also an important cause of UTIs, particularly in the nosocomial setting. This project will use a blend of genetics, high-resolution electron and immunoflourescent :microscopy, mutagenesis and well-established in vitro tissue culture and in vivo mouse cystitis models to gain an understanding of the bacterial factors which are involved in the molecular cross-talk between the host and the pathogen. This interaction determines whether an infection occurs and if the infection is quickly cleared with a return to sterility in the bladder or a persistent reservoir results. The genetic differences in sequence and regulation between K12 and clinical strains of E. coil (both symptomatic and ASB) will be determined, using sequencing and microarrays. These studies will provide the basis for both targeted and global mutagenesis analyses. The mechanisms by which UPEC proceeds through each step of pathogenesis will then be determined and the contributions of specific bacterial genes to virulence will be determined. The triggers for innate host defenses, specifically the interactions between bacterial LPS with the host TLR4 signaling system will be analyzed. In addition, mutations that are developed in this Project, that are deficient in pathogenesis will be used in other projects to gain a detailed understanding of the host response and the molecular cross-talk that occurs as a consequence of host-pathogen interactions. These studies will provide insight into possible downstream sequelae, such as recurrent UTIs. The knowledge of general and specific mechanisms of infection will be increased by investigating the molecular basis of how Enterococcus interacts with the urinary tract and causes disease. The specific knowledge that will be gained in this project will: i. lead to a better understanding of the mechanisms by which bacteria causes acute, recurrent and chronic cystitis, ii. lead to improved methods of treating and preventing this ubiquitous disease and iii. establish a coordinated set of analyses and model systems which can be used to understand the fine molecular details of both the early and long-term consequences of the interaction between a pathogen and its host tissue. In addition, since a wide variety of diseases are caused by Gram-negative bacteria that infect or invade mucous surfaces, the knowledge gained will be applicable to many infectious diseases.

Principal Investigator: Thomas M. Hooton, M.D.

Project 2: Microbial Reservoirs and UTI in Women

Acute uncomplicated urinary tract infections (UTIs) occur in an estimated 7-11 million women each year, and the annual costs of caring for these women are thought to approach $1.6 billion. Approximately 20-30% of women suffer from frequent recurrent infections. UTIs in young women result in substantial morbidity, time lost from work, and medical costs. An improved understanding of the pathogenic mechanisms underlying UTIs could result in novel approaches to their prevention and reduced morbidity and antimicrobial use. In this project we seek a better understanding of the pathogenesis of UTI. The widely accepted model of UTI pathogenesis is that vaginal colonization with uropathogens from the rectal flora precedes urethral and bladder colonization and subsequent UTI, but the temporal relationship between these events has not been established and some women with UTI cannot be found to harbor vaginal uropathogens. Moreover, recent data from studies in mice strongly suggest that persistent bladder epithelial infection follows an initial bladder infection. In this project, we will prospectively follow a large cohort of women with recurrent UTI to determine 1) the temporal relationships between vaginal colonization with a uropathogen, asymptomatic bacteriuria and symptomatic UTI and 2) the presence of persistent bladder epithelial infection following symptomatic UTI and its relationship to same-strain recurrence of UTI. We will thus be able to determine the relative importance of vaginal colonization vs. persistent infection of the bladder epithelium as the origin of the infecting strain in same-strain recurrent UTI. Uropathogenic strains isolated from well-characterized episodes of UTI and asymptomatic bacteriuria will undergo genotyping in Project 1 to identify unique genes or gene dusters that may contribute to their clinical phenotype. The molecular pathogenesis of representative strains will also be examined in Project 1 and their effect on host response will be determined by functional genomic analysis in Project 3. A better understanding of the molecular and epidemiologic basis of UTI is critical in developing optimal prevention and management strategies.

Principal Investigator: Jeffrey I. Gordon, M.D.

Project 3: Functional Genomic Studies of Urinary Tract Infection

We have used functional genomics (i.e., DNA microarrays with follow-up real time quantitative RT-PCR and in situ hybridization analyses) to examine the bladder responses of adult C57B1/6 female mice during the first steps of infection with virulent (FimH+) and isogenic avimlent (FimH-) UPEC strains. Project 3 will extend these analyses with four specific aims. (1) Further characterize the evolution of the response of the adult female C57B1/6 mouse bladder to infection with two genotyped clinical isolates. Our previous studies focused on the acute phase of infection with the cystitis isolate NU14. We will now use DNA microarrays to profile gene expression in the bladders of adult female C57B1/6 mice from 1.5h to 6 weeks after infection with UTI89 (a virulent strain whose genome will be sequenced in Project 1), and a genotyped asymptomatic bacteriuria (ASB) strain. The temporal evolution as well as the cellular origins of selected responses will be defined using real time quantitative (q) RT-PCR, laser capture microdissection (LCM), in situ hybridization, and multi-label immunohistochemical analyses. (2) Define the impact of specified UPEC genes on the response of the adult female C57B1/6 mouse bladder to infection. We will examine host responses to isogenic strains of UPEC that have engineered mutations in genes expected to alter various steps in the proposed 6 step pathogenic cascade. These mutants will be generated in Project 1 and will include (el) genes targeted based on results obtained from the UT 189 genome sequence, results obtained from differential genotyping of clinical strains, and results garnered from studies of bacterial gene regulation; (b) genes identified during screens of a random transposon-tagged library and (c) mutants in already suspected virulence factors. (3) Define the response of the adult female C57B1/6 mouse bladder to a Gram-positive uropathogen. Enterococcus spp are an important cause of nosocomial infection. A functional genomics-based comparison of the responses to infection with isogenic wild type and fimH strains of an Enterococcus faecalis cystitis isolate will provide insights about how the bladder responds to attachment of Gram-positive uropathegens (4) Define host genes that regulate responses to infection with isogenic wild type and mutant strains of UPEC and E. faecalis. Toll-like receptor (TLR) pathways are involved in the response to infections by both Gram-negative and Gram-positive bacteria. We will perform a comparative functional genomics analysis of the host response to FimH+ UPEC and FimH + E. faecalis in adult female C57B1/6 mice homozygous for wild type tlr alleles, versus the responses of C57B1/6 mice homozygous for tlr4 or tlr2 nuU alleles. We will also examine the contributions of inducible nitric oxide synthase (iNOS), matrilysin (MMP-7) and A20 by infecting genetically engineered C57B1/6 mice homozygous for null alleles of these genes with wild type and mutant strains of bacteria characterized in aims 2 and 3.